



## ***Plasmodium vivax* Detected on *Anopheles punctulatus* Using ELISA: A Vector Confirmation Study in Tamraw District, A Remote Area With High Incidence of Malaria, West Papua Province-Indonesia**

Authors

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### **Abstract**

**Background:** *Tamraw District is a new administrative district of West Papua Province with high incidence rate of malaria. The aim of the study was to detect Plasmodium sp in Anopheles sp using ELISA assay in Tamraw District.*

**Material and Methods:** *Mosquitoes sample collection was carried out using human landing collection method in Anopheles sp active period (18.00-06.00). The identification of species was done in situ, immediately after collection. The sandwich ELISA for detection *P.falciparum* and *P.vivax* sporozoit was carried out.*

**Result:** *The total Anopheles punctulatus groups collected in this study were 179, consist of An. farauti (15%), An.koliensis (7%) and An.punctulatus (78%). The active period peak of Anopheles punctulatus group was on 19.00-20.00 in indoor area. The ELISA result shows that Plasmodium vivax was detected on of Anopheles punctulatus samples with the sporozoit index 3,27%.*

**Conclusion:** *Base on sporozoit detection using ELISA, Anopheles punctulatus in Tamraw District West Papua confirmed as a malaria vector.*

**Keywords:** *Plasmodium vivax, ELISA, malaria, Anopheles punctulatus*

### **INTRODUCTION**

Malaria is a life-threatening disease in Indonesia which is transmitted by Anopheles mosquitoes. Twenty four Anopheles species recorded in Indonesia, ten are major malaria vectors<sup>(1)</sup> Basic Health Research revealed that malaria prevalence in West Papua was 33,8% and become the highest number in Indonesia.<sup>(2)</sup>

The *Anopheles punctulatus* group contains the major malaria vectors Papua.<sup>(3)</sup> Other research in

Timika Papua Province, revealed the dominant malaria vector in this area were: *Anopheles punctulatus* complex (*An. punctulatus*, *An. farauti* dan *An. Koliensis*) with *P.falciparum* and *P. vivax* preference.<sup>(4),(5)</sup> The ability of the mosquitoes to transmit *Plasmodium spp.* is dependent upon a series of biological features generally referred to as vectorial capacity<sup>(6)</sup> which is influenced by anthropophilic behavior and *Plasmodium* existence in mosquito's salivary gland<sup>(7)</sup>.

Tamraw District was located in Bird's Head Peninsula Papua Province (0°4'00''-1°4'00'' South dan 131°07'00 - 131°56'00 East) with forest, coastal and hill ecosystem area. This area had high malaria cases. There are no data about Anopheles species in Tamraw District. Data about Anopheles sp as malaria vector is very important for deciding the best malaria control method in a area base on vector confirmation and its behavior. Aims of this study were to detected the plasmodium and identified the preference on vector suspected mosquitoes in Tamraw District West Papua Province.

## METHOD

This research was a cross sectional descriptive study. Entomology survey was carried out in May and June 2013. Adult mosquitoes were collected indoor as well as outdoor using human landing collection method in four different houses per village from 06:00 p.m. to 06:00 a.m. One period of collection was 50 minutes for collection and 10 minutes for rest. Two collectors captured a mosquito landed on the bodies using aspirator and placed the samples to the paper cup. The ecosystems for collecting the data were hill (Kwor and Bikar village) and beach (Sobey and Hopmare). Larvae collection was done to determine location of breeding habitat using 350 ml deeper.

The identified mosquito was sectioned to determine the parousity. Parous rate determination was performed to determine the age of the mosquitoes. It was determined using the formula:

$$\text{Parousity rate} = \frac{\text{Number of parous}}{\text{Number of dissected mosquito}}$$

Detection of Plasmodium in adult mosquitoes was done using sandwich ELISA. The mosquitoes were sectioned to separate the thorax and head from their abdomen. Sporozoite ELISA was done using 5 individual mosquitoes in one pooling. The pool samples were mixed with 100 ul Casein Blocking Buffer (Sigma) and grinded using pellet

paste. ELISA plate was coated with 50 ul *Plasmodium vivax* Monoclonal Antibody (PV 210 KA 52-5 KPL) in concentration 0,25 ul / 50 ul. Plate than was incubated overnight. After incubation, plate was aspirated and filled with Casein Blocking Buffer 200 ul per well and incubated for 1 hour. The wells were aspirated from Casein Blocking Buffer and fill with 50 ul grinded samples contain the head and thorax. Beside the samples, the positive control (*Plasmodium vivax* Pv 210 – KPL) and negative control (mosquitoes adult from rerange larva in entomology laboratory) were loaded in plate in 8 level concentration ( 500 ng-2,5 pg/ ul). The incubation was done for 2 hours. The process was continued by aspirated the well and washed twice using PBS-Tween( 0,5 ml Tween 20 Sigma in 1 L PBS 1x Dubelco). The wells was filled with Mab-peroxidase conjugate PV(0,05 ug Peroxidase labelled PV 210 KA 51-5 KPL in 50 ul Casein Blocking Buffer) than incubated. After one hour, wells were washed 3 times using PBS-Tween and filled with 100 ul substrate solutions (KPL). The reading was caried out after 1 hour incubation after adding substrat. The wells that changed in color visually was indicated that the sporozoite is detected in mosquito samples.

The same ELISA steps was also conducted to determine the mosquito blood feeding preference between human or animal that is expressed in Human Blood Index (HBI), with the differences in type of samples, monoclonal antibody reagent and peroxidase labeled reagent. The procedure of blood feeding ELISA was done using abdomen samples that contain the blood (fed or half gravid mosquitoes). Affinity purified antibody to human IgG (H+L) (KPL 01-10-06) was used as Monoclonal antibody captured while the product from same company (KPL 074-1006) was used for peroxidase labeled for Antibody to human IgG.

The formulae in malaria epidemiology are reviewed that relate entomological parameters to malaria transmission, including mosquito survivorship and age-at-infection, the stability

index (S), the human blood index (HBI), proportion of infected mosquitoes, the sporozoite rate, the entomological inoculation rate (EIR), vectorial capacity (C) and the basic reproductive number (R0).<sup>(8)</sup>

The transmission potential in an ecosystem was measure using Vector Capacity formula (6) :  $VC = ma^2.p^n / -\ln(p)$

The research was conducted by approval from ethics committee National Institute of Health Research and Development, Ministry of Health Republic of Indonesia.

**RESULT**

Mosquitoes data from in Tambraw District are presented in Table 1. The total number of mosquitoes were 179, consist of *An.farauti*, *An.koliensis* and *An.punctulatus*. The parosity rate is 0,66.

Observation of bionomicis necessary to determine the period of biting activity of *Anopheles punctulatus* group. This data is presented in Figure 1. Figure 1 shows that the peak of activity of the mosquitoes is at 22:00 to 23:00 and 1:00 a.m. to 02:00. The biting activity on the outdoor is higher than on the indoor.

Larvae survey for study breeding habitat of *Anopheles punctulatus* group revealed that the

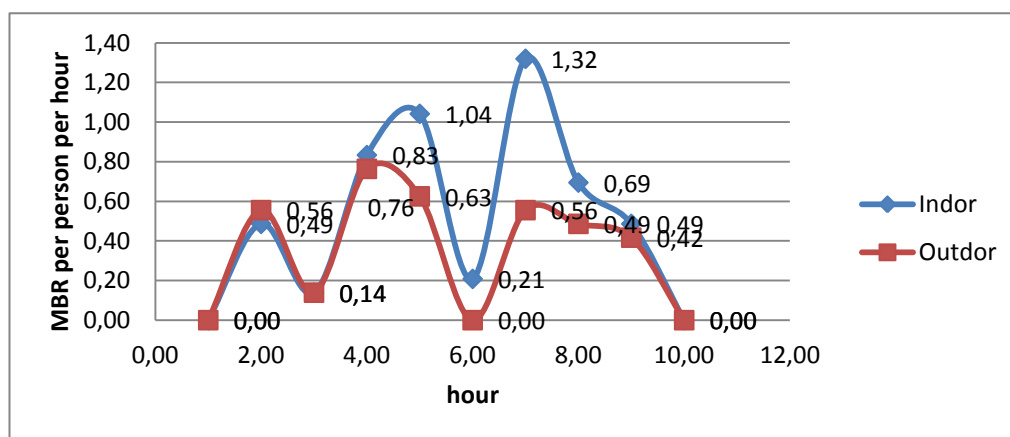
breeding habitat of them are former escavator, ship, ditch and sago palm fronds. The data of Elisa test shows that the sporozoites was detected on *An.punctulatus* on number 5 individuals from 153 mosquitoes tested. Mosquitoes are positive for *P. vivax* sporozoites found in the Bikar Village on the hour between 19:00 to 20:00 from outdoor . Results of the analysis of entomology parameters are presented in Table 2.

**Tabel 1.** Species and number of *Anopheles*

Species	Number
<i>An.farauti</i>	27
<i>An.koliensis</i>	12
<i>An.punctulatu</i> <i>s</i>	140
Total	179

**Table 2.** Parameters of Entomology

Parameters	Result
Vectorial capacity (VC)	106,27
Sporozoit Index (SPI)	3,27
Entomological Inoculation Rate (EIR)	40,16
Stability Index (SI)	2,26



**Figure1.** Biting Activity *Anopheles punctulatus* group Indoor and Outdoor in Tambraw District

## DISCUSSION

Health Basic Research data in 2007 shows the prevalence of clinical malaria in the province of West Papua is the highest in Indonesia with the percentage of 26,1 %.<sup>(9)</sup> Problems of malaria control are also faced because of division of administrative regions so that the land was opened for settlement. Tambraw District which is the area with the expansion of health infrastructure that is still in development.

*An.punctulatus* is the most abundant species in this study found in Kampung Bikar Village. This village is an isolated plateau region from the nearest city center namely Sausapore. Access to the location reached by road with inadequate infrastructure. *Plasmodium vivax* are detected in *An.punctulatus* found in Kampung Bikar. Ovarian surgery also showed that 0.66 *An.punctulatus* group of species in a state of parous. Parous condition which indicates that mosquitoes are already experiencing a phase of egg production allows mosquito vectors of malaria. *Punctulatus* group has been reported as a vector in the South Pacific and 3 species in this group successfully collected in Papua New Guini<sup>(10)(11)</sup>.

Vectorial capacity is forecast transmission index reflected as a mean forecast inoculation of one case of malaria transmission in units of time. Vectorial capacity is determined by the time sporogoni, vector density and time of infection in humans.<sup>(12)</sup> To maintain endemicity in an area required 0.01-0.03 vectorial capacity. In this study Tambraw District vectorial capacity of 106.27. A key factor of stability and transmission of malaria endemicity is the large mosquito populations and age mosquito enough to transmit malaria. There is a tendency of the vector mosquito to die because of the influence of outside factors before finalizing the period of his life. Only a portion of mosquito vectors long-lived and have enough time to transmit pathogens. Vector mosquitoes suck blood every 2-4 days with the period between the time of blood sampling is highly dependent on the temperature and the vector A human bite. In this study it was found that the preference of

mosquitoes biting humans are very large with Human Blood Index of 79,08.

Entomological inoculation rate (EIR) is a value that indicates the estimated infection risk of malaria vector in the study area.<sup>(13),(14)</sup> There are 3 EIR categories, namely: low intensity of transmission (EIR 1-10), medium intensity transmission (EIR 11-100) and high transmission intensity (EIR 101-1000). In the research Tambraw District is categorized in medium intensity transmission with the EIR value is 40,16. Stability Index (SI) is the index that describes the stability of malaria transmission in an area. This is classified into 2 categories, namely: Indication stability of malaria transmission in the study area if the value of  $> 2.5$  and indications unstable if the value of  $< 0,5$ . Based on SI Tambraw district indicates unstable malaria transmission with the value 2,26.

Based on the analysis parameters which include VC, EIR, SI and SPI, it is necessary to surveillance and intervention, especially for Tambraw District. Interventions that can be recommended is public education to tribal languages and using media that is easy to understand, the application netting in the house with regular monitoring and evaluation to deny the failure application<sup>(15)</sup>. Active Case Finding in the form of Mass Blood Survey needs to be done to screen for malaria.

## CONCLUSION

In this study *Anopheles punctulatus* in the Tambraw district is confirmed as vectors that transmit *Plasmodium vivax* using ELISA test.

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